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Near Infra-red Labels for Arrayed Colony Filter Hybridization

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The Bio-instrumentation group at LLNL has been an active participant in the Human Genome Project, and is developing methods and equipment for high throughput, non-radioactive hybridization techniques for screening of high-density arrayed genomic and cDNA clones. Our long term goal is to develop the technology necessary for probing one million clones (about a 10x coverage of the human genome in BAC-size clones) with multiple probes over a period of a few years. In this poster we will describe recent progress in the use of near-IR fluorescent probes and associated detection systems for colony filter hybridization.

We continue to use our custom one meter by two meter gantry robot with a unique 384-pin tool for producing high density arrays. The spotting robot is being used to produce arrays of 13,824 clones on an 8-cm x12-cm nylon substrate.

Near-IR probe labeling methods are being optimized for use in hybridizations against cosmid and BAC colony filters on nylon. Infra-red fluorescent probes, generated by PCR from a number of different sources including bacterial suspension, have been successfully hybridized and detected.

For probe detection, we have recently started using a modified LI-COR (LI-COR Inc., Lincoln, Nebraska) sequencer as an infra-red scanner. The converted sequencer uses a microscope head which travels across a fixed scan window. We have added a second axis to allow filter movement perpendicular to the scan window for collection of 2-dimensional scan data. The prototype instrument scans two eight by twelve centimeter filter membranes in less than ten minutes. The system's optical resolution is about two hundred microns in diameter and the fluorescence detection limit is about thirty attomoles, or one dye molecule per square micron.

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